ADAM

rWBC

New standard of automatic residual leukocyte counting
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What is ADAM-rWBC?

ADAM-rWBC is an analyzing device that counts the number of residual leukocytes in a blood component for transfusion.

Targeting the leukocyte, the ADAM-rWBC uses fluorescent dye to perform cell dyeing and automated cell counting.

Automated cell counting eliminates user bias or subjective interpretation that can be found when counting residual leukocytes using other methods.

3 Minutes, You can count on!
Development history

• 2008 Start to develop accurate cell counter ADAM series

• 2009 Developed prototype of ADAM-rWBC

• 2010 AABB participation

• 2012 ISBT-AABB participation

• 2012 FDA 510k cleared

Advancing Transfusion and Cellular Therapies Worldwide
Leucodepletion guideline

• Leucodepletion of blood products prevent to
  - HLA alloimmunization, Platelet refractoriness, Febrile reactions
  - CMV (Cytomegalovirus) & HTLV-1 (Human T-lymphotropic virus) infection,

• Sample must be prepared by a method known to reduce the
  Leukocyte number to <5 x 10^6

• The sampling plan should include testing a minimum of 1% of the
  establishment’s monthly production of each leukocyte-reduced blood
  product (Whole Blood, Red Blood Cells, Platelets, or Platelets, Pheresis)

Source: AABB standard 5.7.4.1 and FDA memorandum May 29, 1996; Recommendations and Licensing requirements for
leukocyte-reduced blood products
Intended use

• ADAM-rWBC is designed for counting residual white blood cells (Leukocyte) in leukoreduced blood products

• How can we assure that white blood cells have been filtered out properly?
Blood component work flow

Blood donation → Whole blood → Apheresis platelets → Apheresis RBC → RBC → Platelet → Serum → coag. factor → Process of filtering Blood pack → rWBC Counting
Existing method for rWBC counting

**Manual counting**

Price: $0 / Time: 20-30 min / Labor cost: $ 10
✓ Chamber & reagent required

**Flow Cytometry**

Price: $20 / Time: 5-10 min / Labor cost: $4
✓ Additional costs required
  • sample transport cost
  • instrument maintenance cost
  • need 24 hr more to get the test results by FACS
New solution for rWBC counting

Accurate result
- $r^2 = 0.989$
- Substantially equivalent to flow cytometry

Fast measurement
- Mix sample with staining sol.(PI) simply
- 3 minutes for counting rWBC

Easy to use
- Only 3 step for operate system
- User friendly interface

• The ADAM-rWBC is an alternative method to Nageotte and flow cytometry
Principles of procedure

- The ADAM-rWBC utilizes sensitive fluorescence dye staining, LED excitation and CCD detection technologies makes the WBC analysis more accurate and reliable.
Principles of procedure

• The ADAM-rWBC automatically focuses on the slide. The stained cells are taken and recorded by a sensitive CCD camera. The ADAM-rWBC analyzes and reports a result in using integral image analysis software.

• ADAM-rWBC counts the cells 203 images and averages out the counting results to increase the accuracy and reliability
How to use

• Adam-rWBC Procedure is very simple and easy so that everyone can use it.

100 µL

400 µL

Step 1: Sample preparation (diluted 1:5 ratio with r-solution)

Step 2: Sample loading & inserting

Step 3: Get the result (3 min)

Total Cell: 5.2 / µl
Performance characteristics

Accuracy (ADAM-rWBC vs. BD Leucocount kit)

Red blood cell products

![Graph showing linearity for red blood cell products.](image)

- Slope = 1.010
- Intercept = 0.299
- r² = 0.989

Platelet products

![Graph showing linearity for platelet products.](image)

- Slope = 1.027
- Intercept = 1.066
- r² = 0.989

Linearity (ADAM-rWBC vs. BD Leucocount kit)

Red blood cell products

![Graph showing linearity for red blood cell products.](image)

- Slope = 1.058
- r² = 0.988

Platelet products

![Graph showing linearity for platelet products.](image)

- Slope = 1.046
- r² = 0.990
Components/Calibration/Storage

Calibration

Standard Bead Solution is used
• to provide absolute particle numbers
• to calibrate the automatic focus
• to check the position of the slide stage

Storage & Handling

• r-Solution : 2 - 8°C
• Standard Bead: 2 - 8°C
• r-Slides : 20 - 25°C

※ Expiration date
• r-Solution and standard bead : 1 year before open, 6 month after open
• r-Slides: 2 year
FDA approval

• Special 510(k) Summary

A) SUBMITTED BY: NanoEnTek, Inc.

SPONSOR: 300 Washington St, STE 416 Newton, MA, 02458, USA

CONTACT: Sharyn Orton, PhD
MEDIcept Inc. 200 Homer Ave Ashland, MA 01721 401-330-8264 508-231-8861

B) DEVICE NAME: ADAM- rWBC
COMMON NAME: Automatic Cell Counting System
DEVICE CLASS: 21 CFR 864.5220, Automated Differential Cell Counting System  Class II
PRODUCT CODE: GKZ

C) PREDICATE:
BK120015 ADAM –rWBC Automatic Cell Counting System
Certificates

CE: ADAM rWBC Device

CE: ADAM rWBC Kit

Certificate Of GMP

ISO13485 Certificate
Who use the ADAM-rWBC in US?

Walter Reed National Military Medical Center supply the blood after QC using ADAM-rWBC for Barack Obama (RH-AB).

After QC with ADAM-rWBC, blood supplies to US naval hospital and Okinawa US marine corps.
<table>
<thead>
<tr>
<th>No</th>
<th>Customer</th>
<th>Website</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WALTER REED NAT. MIL. MED. CTR (WRNMMC)</td>
<td><a href="http://www.wrnmmc.capmed.mil">www.wrnmmc.capmed.mil</a></td>
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<tr>
<td>2</td>
<td>US Naval Hospital, Guam</td>
<td><a href="http://www.med.navy.mil/sites/usnhguam">www.med.navy.mil/sites/usnhguam</a></td>
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<tr>
<td>3</td>
<td>US Naval Hospital Okinawa</td>
<td><a href="http://www.med.navy.mil/sites/nhoki">www.med.navy.mil/sites/nhoki</a></td>
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<td>4</td>
<td>Children's national Med. CTR</td>
<td>childrensnational.org</td>
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<td>5</td>
<td>Blood Bank of Hawaii</td>
<td><a href="http://www.bbh.org">www.bbh.org</a></td>
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<tr>
<td>6</td>
<td>UCLA Blood Bank</td>
<td>gotblood.ucla.edu</td>
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<tr>
<td>7</td>
<td>Canadian Blood Services</td>
<td><a href="http://www.blood.ca">www.blood.ca</a></td>
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<tr>
<td>8</td>
<td>Memorial Sloan Kettering Cancer Center</td>
<td><a href="http://www.mskcc.org">www.mskcc.org</a></td>
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<tr>
<td>9</td>
<td>San Diego Blood Bank</td>
<td><a href="http://www.sandiegobloodbank.org/">www.sandiegobloodbank.org/</a></td>
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<tr>
<td>10</td>
<td>Mayo Foundation</td>
<td><a href="http://www.mayoclinic.org/">www.mayoclinic.org/</a></td>
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</tbody>
</table>

100 units sold
The leading organization of board-certified pathologists serves patients, pathologists, and the public by fostering and advocating excellence in the practice of pathology and laboratory medicine worldwide.

- Established in 1946, a certification system of U.S. private organization
- 7,000 research institutes and 22,000 laboratories participate the test around the world
- CLIA 88 (quality requirement law of clinical test), authorized system by CDC
- Blind test (use sample that result value is hidden)
- Publish the result of cap survey once a year
- Reliability of the U.S. FDA
- Verify blood management condition, accuracy of product and proficiency of employee

* In 2013 & 2014, ADAM-rWBC passed cap survey, and verified higher accuracy than FACS
Cap Survey Result (2013 & 2014)

Cap Survey announced recently,

100% ADAM-rWBC Users passed 2013 & 2014 CAP Survey Test,
while FACS users and Nageotte users passed respectively 98% and 74% in 2013.

<table>
<thead>
<tr>
<th></th>
<th>2013 RBC</th>
<th>2013 PLT</th>
<th>2014 RBC</th>
<th>2014 PLT</th>
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</thead>
<tbody>
<tr>
<td>2013 RBC</td>
<td>100.0%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2013 PLT</td>
<td></td>
<td>98.7%</td>
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<td></td>
</tr>
<tr>
<td>2014 RBC</td>
<td></td>
<td></td>
<td>97.0%</td>
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<tr>
<td>2014 PLT</td>
<td></td>
<td></td>
<td></td>
<td>98.5%</td>
</tr>
</tbody>
</table>

100% Accuracy

ADAM-rWBC FACS Nageotte

All 100%

360% Up!!

CAP survey participation using ADAM-rWBC

100% ADAM-rWBC User Pass
Cap Survey Result (2013 & 2014)

TRC-C

Transfusion-Related Cell Count

The sample for residual leukocyte count for the red cell product TRC-25 was intended to pass leukocyte reduction criteria with intended residual WBC content in a 300 mL product of <5 x 10^6 WBCs. The bulk testing of the proficiency sample by flow cytometry prior to shipment demonstrated 0.60 x 10^6 WBCs per 300 mL product.

The intended result was obtained by 100% of participants utilizing flow cytometry with a mean number of WBCs of 0.442 x 10^6. Similarly, 100% of participants utilizing fluorescence microscopy obtained the intended result with a mean number of WBCs of 0.449 x 10^6. When Nageotte chambers were utilized, 97.0% of participants correctly identified the units as being leukocyte reduced. Two participants utilizing the Nageotte chamber indicated that TRC-25 was not leukocyte reduced. However, the highest reported residual leukocyte count for users of the Nageotte chambers was 1.7 x 10^6. This value is below the leukocyte reduction standard of <5 x 10^6 indicated in the instructions to the participants.

The sample for residual leukocyte count on platelet product TRC-27 was intended to pass leukocyte reduction criteria with intended residual WBC content in a 300 mL product of <5 x 10^6 WBCs. The intended results were obtained by 100% of participants utilizing flow cytometry, 100% of participants utilizing fluorescence microscopy, and 98.5% of participants utilizing Nageotte chambers. The highest reported residual leukocyte count for users of the Nageotte chambers was 1.50 x 10^6. This value is below the leukocyte reduction standard of <5 x 10^6 indicated in the instructions to the participants.
References

• Oral presentation in AABB 2012

Oral Presentation to be given on Monday, October 8th at 5:15 PM, Product Manufacturing Session (030E)


Linda Kline¹, Ginny George², Sharyn Orton¹, Jed Gorlin¹, Mark Janzen¹, Pamela Whitley³, Michael Wellington⁴, Jaye Brodsky⁵

¹ American Red Cross Holland Laboratory, Rockville, MD
² MEDcept Inc., Ashland, MA
³ Memorial Blood Centers, St. Paul, MN
⁴ American Red Cross Mid-Atlantic Region, Norfolk, VA
⁵ Quality Analytics, Inc., Riverwoods, IL

Linearity

• At each site 1 RBC and 1 PC were spiked with target concentrations of 0–1, 5–10, 20–30, 50–60 and 80–100 WBC/ul.
• Three replicates of each stained sample were tested using a 4 minute settling time.

Conclusion: RBC & PC

• Stain to Stain Precision
  • %CVs consistent with Repeatability %CVs.
• Linearity
  • Demonstrated to be between 1 and 100 WBC/ul.
• Accuracy
  • The ADAM-rWBC system correlates well with and is substantially equivalent to Leucocount.
  • A settling time of 4 to 7 minutes can be used.
• Stained and Stored Sample Stability
  • Stained samples can be held at RT for up to 1 hour.
  • RBC samples can be held at 1–8°C and PC samples held at 1–8°C or RT; both for up to 24 hours prior to staining and testing.

The ADAM-rWBC system has recently been FDA cleared.
References

- External control package insert

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameter</th>
<th>Low LOT</th>
<th>Low LKR073L</th>
<th>High LOT</th>
<th>High LKR073H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Cytometer</td>
<td>WBC/uL</td>
<td>2.2</td>
<td>0.7 – 3.7</td>
<td>22.0</td>
<td>16.0 – 28.0</td>
</tr>
<tr>
<td>Nageotte Chamber</td>
<td>WBC/uL</td>
<td>2.0</td>
<td>0.5 – 3.5</td>
<td>18.0</td>
<td>12.6 – 23.4</td>
</tr>
<tr>
<td><strong>ADAM rWBC</strong></td>
<td>WBC/uL</td>
<td>2.0</td>
<td>0.2 – 3.8</td>
<td>20.0</td>
<td>13.0 – 27.0</td>
</tr>
</tbody>
</table>
References

• Publication of ISBT journal

**Vox Sanguinis**

**ORIGINAL PAPER**

Comparison of a new microscopic system for the measurement of residual leucocytes in apheresis platelets with flow cytometry and manual counting


Department of Transfusion Medicine and Haemostaseology, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean cell concentration ± SD per µl</th>
<th>Minimum-maximum cell concentration per µl</th>
<th>P value compared to Nageotte</th>
<th>P value of ADAM-rWBC compared to Flow cytometry</th>
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</thead>
<tbody>
<tr>
<td>Nageotte</td>
<td>0.0725 ± 0.088</td>
<td>0.0-0.3</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Flow cytometry</td>
<td>0.470 ± 0.579</td>
<td>0.0-2.81</td>
<td>0.000318</td>
<td>-</td>
</tr>
<tr>
<td>ADAM-rWBC</td>
<td>0.468 ± 0.417</td>
<td>0.0-2.16</td>
<td>0.0000002</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Table 1 Mean leucocyte concentration and absolute cell count, minimum and maximum leucocyte cell concentration and absolute cell count of 40 APLT units measured by three different methods and P values
Appendix. Specification

**ADAM-rWBC**
- **Voltage:** AC100~240 V, 50~60 Hz
- **Current:** max. 1.8 A, max 100 W
- **Fuse:** F3.15AL250V
- **Objective lens:** 4 X
- **LED:** 4W Green LED
- **CCD camera:** B/W CCD
- **Filter:** Excitation filter, Dichroic filter, Emission filter
- **Weight:** 9 Kg
- **Size (W×L×H):** 220 × 375 × 250 mm
- **Degree of protection:** IPX0

**r-Slide**
- **Measuring range:** 1 ~ 100 cells/μl
- **Analysis time:** 2.5 ~ 3 min/test
- **Loading sample vol.:** 100 μL/r-Slide
- **Measuring vol.:** 56.73 μL/r-Slide

**r-Solution**
PI (Propidium Iodide) staining of leucocytes.

**External video monitor**
(Fuse: 250 VAC, 3 A; F3.15AL250V)

**Accessories**
- **Barcode reader:** LS2208 (optional)
- **Color:** Twilight black, Cash register white
- **Corded:** Yes
- **Dimensions:**
  - 6 in. H x 2.5 in. W x 3.34 in.
  - D 15.2 cm H x 6.3 cm W x 8.4 cm
- **Handheld:** Yes
- **Power source:**
  - Host power or external power supply; depends on host type
- **Voltage and current:**
  - 5 volts +/- 10% at 130 mA typical, 175 mA max
- **Weight:** 5.15 oz./146 gm.